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Histologic Effects of Ruby Laser Hair Removal in Japanese Patients

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Background and Objective: **Hair removal by lasers has recently become a popular method to remove unwanted hair. However, histologic changes in human skin before and after exposure to lasers have not been thoroughly investigated. The aim of this study was to clarify the differences that occur immediately after laser exposure and 1 month after laser exposure.**
Study Design/Materials and Methods: **Eight adult Japanese volunteers were recruited for this study. They were treated with a long pulsed ruby laser at 20 J/cm^2. A single 3-mm punch biopsy of the laser-treated sites was obtained immediately after laser irradiation and at the 1-month follow-up visit, and they were analyzed using hematoxylin and eosin, PAM, and immunohistological staining.**
Results: **Immediately after laser exposure, hair follicles were very damaged and had extensive eosinophilic degeneration. One month after laser therapy, one type of hair follicle showed cystlike formations with negative proliferating cell nuclear antigen reactions (PCNA). Another type of hair follicle showed follicular mitotic figures with cytoplasmic halos. Early anagen hair follicles were apparently not treated effectively by ruby laser.**
Conclusion: **Ruby laser leads to extensive follicular damage, and hair follicles considered to be at early anagen phase were not effectively treated. This may be the reason several courses of laser therapy are required to obtain satisfactory results. Lasers Surg. Med. 25:451–455, 1999.** **© 1999 Wiley-Liss, Inc.**

Key words: ruby laser; hair removal; histology

INTRODUCTION

Plucking, waxing, and electrolysis are common methods used to remove unwanted hairs [1–3]. In Japan, depilation is done mostly at esthetic salons, and there is currently no certification required for estheticians. At Japanese esthetic salons, electrolysis is the preferred technique for permanent hair removal [2]. However, electrolysis needs correct needle insertion to each hair follicle [1]; it is tedious and takes a long time for full treatment. Its effectiveness depends on the operator's skills, and regrowth of hair after electrolysis has been reported [2,4].

Recently, hair removal by lasers [5–7] has become popular in the United States and in Eu-

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rope because it is simple, noninvasive, and minimally painful. Compared with electrolysis, it is also faster and more efficient. Ruby [8], Alexandrite [9], Nd:YAG, and argon [10] lasers have been successfully used for hair removal. There are some reports comparing these lasers from clinical and histologic perspectives, but in Japan laser hair removal is not very popular at present. Which types of laser is better for Japanese skin is now under active discussion.

In this study, we examined histologic changes and follicular damage in skin immediately after the hair removal and 1 month exposure.

MATERIALS AND METHODS

Laser Source

A normal-mode ruby laser (Chromos 694, SLS/Biophile, UK) was used. It emits 1-msec pulses at a wavelength of 694 nm up to 0.9 Hz. The aiming beam is a 1-mW HeNe laser delivered by a flexible fiber optic system. The spot size is 5 mm across in diameter.

Patients

Eight healthy adult volunteers (mean age = 32.4 years, range = 22–54 years) were remunerated for participating in this study; informed consent was obtained. All subjects were Japanese and had Fitzpatrick's type IV skin and black hair. Exclusion criteria for this study included chronic illness, cutaneous contact dermatitis, atopic dermatitis, photosensitivity, history of scar formation, history of poor wound healing, or any cutaneous illness.

Hair Removal Procedure

An area was chosen on each patient's tibia, based on the density of terminal hair follicles. Before laser exposure, each site was shaved and then irradiated with the Chromos 694 ruby laser at 20 J/cm^2, with a pulse duration of 1 msec. After exposure, an antibacterial ointment was applied, and patients were instructed to clean the sites gently once a day. Shaving was performed before laser therapy to avoid damaging the lens of the laser.

A 3-mm punch biopsy was taken from each subject immediately after the laser exposure and 1 month later. Specimens were fixed in formalin and were processed routinely, with 4-μm sections being stained with hematoxylin and eosin and PAM. The avidin-biotin complex (ABC) immunoperoxidase method was also used in conjunction with the following antibodies: proliferating cell nuclear antigen (PCNA) for staining cells in the S-phase of the cell cycle (DAKO, Glostrupp, Denmark) [11], S-100 for human S-100 A and B (DAKO) [12], NSE to identify cells of neurogenic origin (DAKO) [13], and αSMA for smooth muscles (DAKO) [14]. Normal skin from the surgical operation material was used as control.

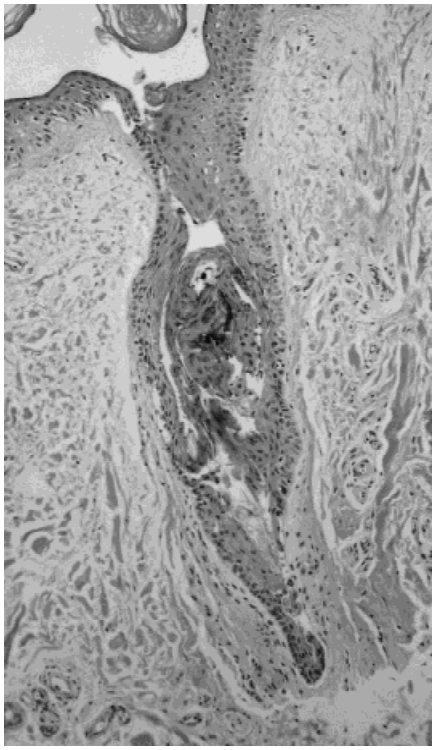

Fig. 1. Hair follicle immediately after laser exposure. Detachment of the focal epidermal–dermal junction and the infundibulum of the hair follicle can be observed. Hair components show marked degeneration. Hematoxylin and eosin, 100×.

This study was approved by the Ethics Committee of Japan Equestrian Federation for Sports Medical Research.

RESULTS

Immediately After Laser Treatment

Clinically, exposure sites showed erythema and scattered mild edema in many cases, which was reduced in 1 week with topical antibiotic use.

Histologically, focal epidermal detachment under basal cells was evident. This detachment developed to the infundibulum of hair follicles. Below the infundibulum, junctions between the outer root sheath and the fibrous root sheath

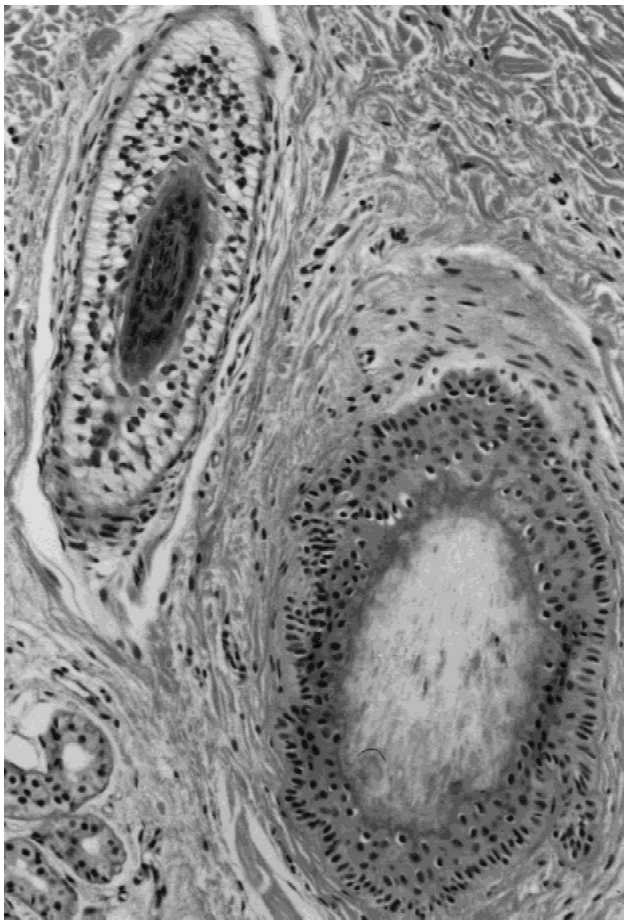

Fig. 2. Two types of hair follicles were observed 1 month after laser treatment. The follicle on the right shows cystlike formations, and follicular epidermal cells on the left show cytoplasmic halos. Hematoxylin and eosin, 100×.

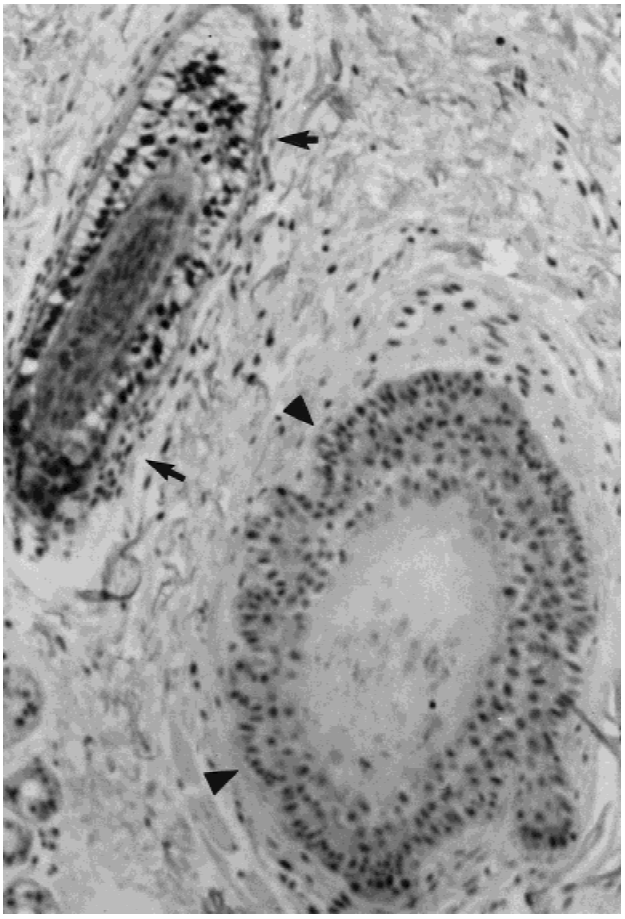

Fig. 3. Correlation of proliferating cell nuclear antigen (PCNA) staining with the histologic appearance of two types of hair follicles. Follicular epidermal cells on the right show negative reaction to PCNA (arrowheads), whereas those on the left show a strong positive reaction (arrows). 100×.

showed partial detachment but were mostly of normal structure. In hair follicles, no hair components were detected. Inner root sheath cells showed eosinophilic degeneration, and they also showed irregular arrangements of cystlike formations. The cytoplasm of the follicular epithelium showed marked eosinophilia (Fig. 1), and in some cases focal rupture of follicles was noted.

One Month After Laser Treatment

Clinically, occasional sparse hairs were observed at a density of 1–2 hairs/cm^2. In four cases, mild pigmentation was evident, but no inflammation or infection was observed.

Histologically, the epidermis appeared normal. The papillary dermis showed marked edematous change with loose collagen structure. Occasional melanin-laden macrophages were observed in the upper to middle dermis. In the middle dermis, perivascular edema was observed, with lymphocyte infiltration.

Two types of hair follicles were observed. One type showed a cystlike formation, and sebaceous glands were observed near this formation in horizontal sections. The epithelium consisted of stratified, large rounded cells, and the cavity showed a dense eosinophilic keratinized mass. There was a mild longitudinal fibrosis around this type, and the nuclei of wall cells showed negative reactions for PCNA.

In the other type of hair follicle, the outer follicular epidermal cells had small nuclei with cytoplasmic halos. These nuclei produced strong positive reactions for PCNA, and occasional mitotic figures were also observed. Inner follicular cells overlying the matrix showed marked eosinophilic platelike structures with no keratinization (Figs. 2, 3).

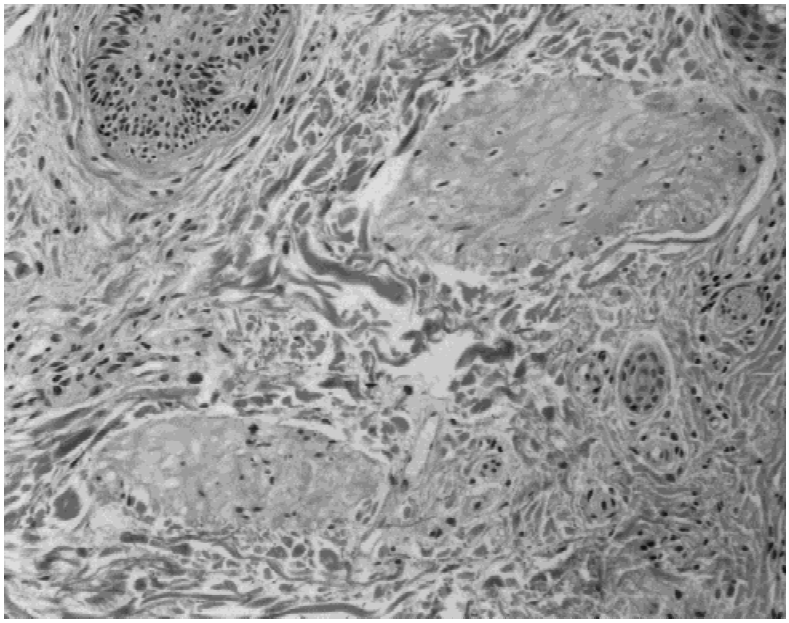

Fig. 4. Nests with spindle cell proliferation were observed in the middle dermis. These spindle cells were strongly positive for NSE, S-100 and αSMA. Hematoxylin and eosin, 100×.

In two cases, nests with spindle cell proliferation were observed in the middle dermis (Fig. 4). Those nests extended to large areas in the dermis and were strongly positive for NSE, S-100, and αSMA. They also produced a moderate positive reaction for PCNA.

DISCUSSION

Long pulsed ruby lasers [5–8,15] have recently been used commercially for hair removal. Laser light targets melanin in the hairs, and the follicles are destroyed along with hair components. There have been some reports about the effectiveness of ruby laser hair removal [16]. Most Japanese have Fitzpatrick's type IV skin, and laser use easily induces Japanese skin to hyperpigment or to form scars [17]. In this study, only mild pigmentation was observed in four cases, but no inflammation or scar formation was evident.

In this study, we examined histologic changes and follicular damage at two stages, immediately after laser hair removal and 1 month later.

Immediately after laser exposure, gaps were seen between the follicular epidermis and the follicular dermis, which also developed at the cutaneous epidermal–dermal junction. Such changes were particularly noticeable at the upper part of the follicle. Grossman et al. [8] reported similar histologic changes in skin from black-haired dogs after laser treatment, but their laser exposures were 40–70 J/cm^2 at 270 μsec. It is currently unknown what exposure parameters are optimal for hair removal.

One month after laser exposure, two types of hair-associated components were observed. One showed cystlike dilated follicular formations, with histologic characteristics very similar to those of trichilemmal cysts. They were considered to be of hair follicle origin because they were near sebaceous glands. Their epithelia produced negative reactions for PCNA. In normal hair follicular cells, there are no follicles with PCNA-negative reaction in any part of the hair cycle. These were considered to be degenerate follicles from anagen phases of the hair cycle [18], and we speculate that these are laser-damaged follicles. In the dermis, scattered melanin-laden macrophages and lymphocytes were observed. These observations are very similar to histologic changes found during wound healing [19], and as in wound healing, these cystic follicles reorganize at a later time.

Other specimens showed no hair shaft keratinization, but their epithelia showed strong reactions for PCNA. These hair follicles are considered to be early anagen phase in view of their mitotic activity and the eosinophilic inner root sheath with no keratinization. These early anagen phase hair follicles may not have been damaged by the laser because they were too young and did not have any melanin components at the time of laser exposure. This may be the reason several treatments are required to complete laser hair removal [5]. This histologic study has proven that

several laser treatments after the regrowth of young follicles are necessary. Grossman et al. [8] reported more than 50% hair regrowth 3 months after laser treatment. In the present study, the effectiveness was almost perfect after 1 month, but these new hair follicles will visibly regrow later. The optimal interval between laser treatments will need to be determined in a future study.

The spindle cell proliferation that was observed in two cases is thought to originate from smooth muscle cells due to their immunohistologic reaction patterns because they were positive for αSMA stain and had a positive reaction to PCNA. They were also positive to NSE and S-100 stains. Their histologic features suggest that they are hair muscle, but hair muscle is usually negative for NSE and S-100 stains and is just weakly positive for PCNA. A similar hyperproliferation has been seen in Paget's disease [20]. It may be that some dermal changes stimulate smooth muscle cells and induce hyperproliferation. However, it is not certain whether this is related to the laser exposure or to the reduction of hair growth.

In this histologic study, it is clear that laser hair removal is a very effective method to destroy hair follicles. However, regrowth of hair in treated areas may result from the survival of early anagen phase hair follicles because lasers can not completely destroy these hair follicles, which are generally unpigmented. The most effective intervals of laser treatment for hair removal therapy will need further study.

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